

CLAIMS

1. A method of determining the assignment of a signal obtained by the NMR measurement of a protein, which comprises:

(i) preparing a protein, wherein the carbon atom(s) at position 2 and/or at position 1 and the nitrogen atom at position 2 of either one of amino acids adjacent to an amino acid to be identified on the amino acid sequence of the protein have been double-labeled for the NMR measurement, and wherein any one of nitrogen, carbon, and hydrogen atoms at position 2 of at least the amino acid to be identified has been labeled for the NMR measurement;

(ii) performing on the above-described protein, the NMR measurement, which is capable of identifying only the correlation signal of the amide proton of an amino acid residue to be identified, adjacent to the double-labeled amino acid, with the labeled atom; and

(iii) comparing the above-described signal with the correlation signal of the amide proton of an amino acid residue to be identified with the labeled atom, which is obtained by the NMR measurement of a protein wherein any one of nitrogen, carbon, and hydrogen atoms at position 2 of the amino acid to be identified has been labeled, so as to determine the assignment of the signal of the amino acid to be identified.

2. A method of determining the assignment of a signal obtained by the NMR measurement of a protein, which comprises:

(i) preparing a protein, wherein the carbon atoms at positions 2 and 1 have been labeled with ^{13}C and the nitrogen atom at position 2 has been labeled with ^{15}N , with respect to either one of amino acids adjacent to an amino acid to be identified on the amino acid sequence of the protein, and wherein the nitrogen atom at position 2 of at least the amino acid to be identified has been labeled with ^{15}N ;

(ii) performing on the above-described protein, the NMR measurement, which is capable of identifying only the correlation signal of the amide proton of an amino acid residue to

be identified, adjacent to the double-labeled amino acid, with ^{15}N ; and

(iii) comparing the above-described signal with the correlation signal of the amide proton of an amino acid residue to be identified with ^{15}N , which is obtained by the NMR measurement of a protein wherein only the nitrogen atom at position 2 of an amino acid to be identified has been labeled with ^{15}N , so as to determine the assignment of the signal of the amino acid to be identified.

3. A method of determining the assignment of a signal obtained by the NMR measurement of a protein, which comprises:

- (a) determining the assignment with respect to either one of amino acids adjacent to an amino acid to be identified on the amino acid sequence of a protein according to the method of claim 2;
- (b) preparing a protein, wherein the carbon atoms at positions 2 and 1 of the above-described amino acid have been labeled with ^{13}C and the nitrogen atom at position 2 thereof has been labeled with ^{15}N , and the nitrogen atom at position 2 of at least the amino acid to be identified has been labeled with ^{15}N ;
- (c) performing on the above-described protein, the NMR measurement, which is capable of identifying only the correlation signal of the ^{13}C of the double-labeled amino acid residue with the amide proton, and the correlation signal of the ^{13}C of the double-labeled amino acid residue with the amide proton of the adjacent amino acid residue to be identified;
- (d) obtaining the correlation signal of the amide protons of the amino acid to be identified and the above double-labeled amino acid with ^{15}N ;
- (e) selecting from the signals obtained in (c) above, a signal having a chemical shift identical to the chemical shift of the amide protons of amino acids, whose assignment has been determined, in the signal obtained in (d) above;
- (f) selecting from the signals obtained in (c) above, a signal having a chemical shift identical to the chemical shift of the ^{13}C of the selected signal; and

(g) selecting from the signals obtained in (c) above, a signal having a chemical shift identical to the chemical shift of the amide proton of the selected signal, and then determining the assignment of the above-described signal, using the fact that the above signal is assigned to an amino acid adjacent to the amino acid whose assignment has been determined.

4. The method according to claim 3, wherein, in step (c), the NMR measurement is further carried out to identify only the correlation signal of the ^{13}C of the double-labeled amino acid residue with the amide proton of the adjacent amino acid residue to be identified, and it is confirmed that the signal selected in step (f) overlaps the signal as obtained above.

5. A method of determining the assignment of a signal obtained by the NMR measurement of a protein, which comprises:

(i) preparing a protein, wherein the carbon atom at positions 1 of either one of amino acids adjacent to an amino acid to be identified on the amino acid sequence of the protein has been labeled with ^{13}C and the nitrogen atoms at position 2 of multiple amino acids including the amino acid to be identified have been labeled with ^{15}N ;

(ii) performing on the above-described protein, the NMR measurement, which is capable of identifying only the correlation signal of the amide proton of the amino acid residue to be identified, adjacent to the ^{13}C -labeled amino acid, with ^{15}N ; and

(iii) comparing the above-described signal with the correlation signal of the amide proton of the amino acid residue to be identified with ^{15}N , which is obtained by the NMR measurement of a protein wherein the nitrogen atom at position 2 of only the amino acid to be identified has been labeled with ^{15}N , so as to determine the assignment of the signal of the amino acid to be identified.

6. A method of determining the assignment of all signals obtained by the NMR measurement of a protein, which comprises repeating the method according to claim 1 or 5, or combining the method according to claim 3 with the method according to claim 4.

7. A method of determining the assignment of the correlation signal of the amide proton with ^{13}C or the amide proton with ^2H , obtained by the NMR measurement of a protein, which comprises:

- (i) determining the assignment of the correlation signal of the amide proton of a protein with ^{15}N by the methods according to any of claims 2 to 6;
- (ii) preparing a protein, wherein the carbon atom(s) or hydrogen atom(s) at position 2 and/or at position 1 of an amino acid to be identified on the amino acid sequence of the above-described protein have been double-labeled for the NMR measurement;
- (iii) obtaining the correlation signal of the amide proton in the amino acid to be identified with the carbon atom(s) or hydrogen atom(s) of the same amino acid, which have been labeled for the NMR measurement, with respect to the above-described protein; and
- (iv) using, as an indicator, the fact that amide protons common in the correlation signal of the amide proton with ^{15}N in (i) above and in the correlation signal of the amide proton with ^{13}C or with ^2H in (iii) above have the same chemical shift, and associating the correlation signal of the amide proton with ^{13}C or with ^2H , with the correlation signal of the above-described amide proton with ^{15}N , so as to determine the assignment of the correlation signal of the amide proton with ^{13}C or with ^2H .

8. A method of identifying the three-dimensional structure of a protein, wherein the chemical shift information of the NMR signal whose assignment has been determined by the method according to claim 6 or 7 is used.

9. A method of identifying the binding site of a protein to a ligand, which comprises: comparing signals obtained by the NMR measurement of a complex of a protein and a specific ligand, with signals obtained by the NMR measurement of only a protein; and determining by the method according to any one of claims 1 to 7, the assignment of a signal whose chemical shift has been changed.

10. A reagent kit used for the method of determining the assignment of signals obtained by the NMR measurement of a protein by the method according to any one of

claims 1 to 7, which comprises: one or more types of amino acids, wherein at least the carbon atoms at positions 2 and 1 have been labeled with ^{13}C and the nitrogen atom at position 2 has been labeled with ^{15}N ; and multiple amino acids wherein the nitrogen atom at position 2 has been labeled with ^{15}N and the carbon atoms at positions 2 and 1 have not been labeled with ^{13}C .

11. A reagent kit used for the method of determining the assignment of signals obtained by the NMR measurement of a protein by the method according to any one of claims 1 to 7, which comprises: one or more types of amino acids, wherein at least the carbon atoms at positions 2 and 1 have been labeled with ^{13}C and the nitrogen atom at position 2 has been labeled with ^{15}N ; multiple amino acids wherein the nitrogen atom at position 2 has been labeled with ^{15}N and the carbon atoms at positions 2 and 1 have not been labeled with ^{13}C ; a wheat germ extract for a cell-free protein synthesis; and an amino acid metabolic enzyme inhibitor.